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Analysis of Urinary Metabolites of Nerve and Blister Chemical Warfare Agents

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Land Division

Defence Science and Technology Organisation

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ABSTRACT

This report outlines the instrumental method development for the analysis of human biofluids, in this case urine, for the presence of metabolites of the chemical warfare agents HD, GA, GB, GD, GE, GF, VX and VR using UHPLC MSMS. It also investigates the effect of different sample preparation techniques on the recovery of the analytes, using this analysis method.

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Analysis of Urinary Metabolites of Nerve and Blister Chemical Warfare Agents

Executive Summary

The analysis of samples from individuals who may have been exposed to Chemical Warfare Agents (CWA) may assist in the triage of persons presenting at health care facilities after a possible exposure. The emphasis of this study was on the rapidity of analysis rather than absolute detection limits, hence we have concentrated on reducing the sample preparation and analysis time.

DSTO has previously developed methods for the analysis of urine for the presence of the metabolites of CWAs. The analysis methods use UHPLC-MS/MS in Multiple Reaction Monitoring (MRM) mode to enhance the selectivity and sensitivity of the method. Although these methods require an instrument capable of acquiring data in this mode, the method could be modified for use on other instruments.

This report outlines the instrumental method development for the analysis of human biofluids, in this case urine, for the presence of metabolites of the chemical warfare agents HD, GA, GB, GD, GE, GF, VX and VR using UHPLC-MS/MS. It also describes the investigation of the effect of different sample preparation techniques on the recovery of the analytes, using this analysis method.

The extraction techniques used for the nerve agent metabolites are all quite simple, though the speed of analysis varies greatly from a matter of about 10 minutes to overnight. The amount recovered also depends greatly on the metabolite in question; for example, the method LOD (Limit of Detection) varied from approximately 3 ppb to 67 ppb and the method LOQ (Limit of Quantitation) from approximately 10 ppb to 270 ppb.

The instrument methods were both rapid and sensitive for the proposed purpose. The LOD for the metabolites of nerve agents was in the range of 2.4 ppb to 3.9 ppb, the LOQ ranged between 7.9 ppb and 13.1 ppb. These results are consistent with results obtained by the laboratory during an Organisation for the Prohibition of Chemical Weapons (OPCW) confidence building exercise held in December 2009. The LOD and LOQ for the sulfur mustard metabolites were not determined, though during the OPCW exercise we were able to detect the metabolite of HD down to high ppt levels.

As the nerve agent metabolites are, typically, the same as the hydrolysis product for that particular nerve agent, these methods are also suitable for the analysis of environmental aqueous samples.

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Stuart graduated with BAppSc from the Royal Melbourne Institute of Applied Science in 1988. Stuart worked at the Victorian College of Pharmacy whilst completing a Masters degree and graduated with a MPharm from Monash University in 1994. Stuart went on to manage the Spec Facility at the Victorian College of Pharmacy for 14 years prior to joining DSTO in 2005. He is applying his background in nuclear magnetic resonance spectroscopy and mass spectrometry to the detection of chemicals of interest to Defence.

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Glossary

ACN	Acetonitrile
CWAs	Chemical Warfare Agents
GA	Tabun, ethyl dimethylphosphoramidate
GB	Sarin, isopropyl methylphosphonofluoridate
GD	Soman, pinacolyl methylphosphonofluoridate
GF	Cyclosarin, cyclohexyl methylphosphonofluoridate
H, HD	Sulfur mustard
HILIC	Hydrophobic Interaction Liquid Chromatography
LCMS	Liquid Chromatography Mass Spectrometry
LOD	Limit Of Detection
LOQ	Limit of Quantitation
MRM	Multiple Reaction Monitoring
MSMS	Tandem mass spectrometry
MSMTESE	1-methylsulfinyl-2-[2-(methylthio)ethylsulfonyl]ethane
OPCW	Organisation for the Prohibition of Chemical Weapons
ppb	Parts per Billion
ppt	Parts per Trillion
QQQ	Triple quadrupole mass spectrometer
SBMTE	1,1'-sulfonylbis(methylthio)ethane
SBMSE	1,1'-sulfonylbis[2-(methylsulfinyl)ethane]
SIM	Selected Ion Monitoring
SPE	Solid Phase Extraction
UHPLC	Ultra High Pressure Liquid Chromatography
VR	Russian VX, S-2-(diethylamino)ethyl O-isobutyl methylphosphonothioate
VX	S-2-(diisopropylamino)ethyl O-ethyl methylphosphonothioate

1. Introduction

The analysis of samples from individuals who may have been exposed to CWAs may assist in the triage of persons presenting at health care facilities after a possible exposure. The emphasis of this study was on the rapidity of analysis rather than absolute detection limits, hence we have concentrated on reducing the sample preparation and analysis time.

During an Organisation for the Prohibition of Chemical Weapons (OPCW) confidence building exercise, the laboratory had previously developed analysis methods for the presence of metabolites of CWAs in urine [1]. Those analysis methods use UHPLC-MS/MS in Multiple Reaction Monitoring (MRM) mode to enhance the selectivity and sensitivity of the method. Although these methods require an instrument capable of acquiring data in this mode, the method could be modified for use on other instruments by fragmenting the molecular ion in the ion source and acquiring data in Selected Ion Monitoring (SIM) mode.

The agents and their corresponding metabolites under study in this report are listed in the following Tables 1 and 2.

Table 1: Nerve agents and their metabolites

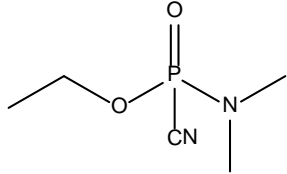
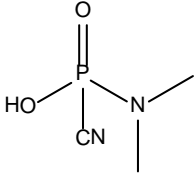
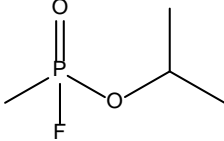
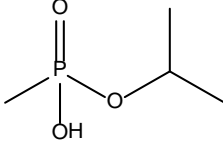
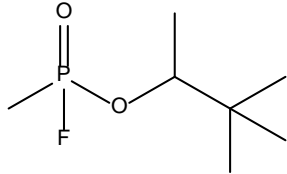
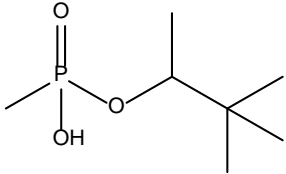
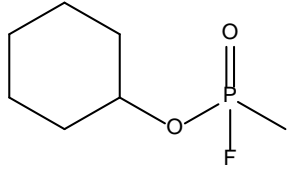
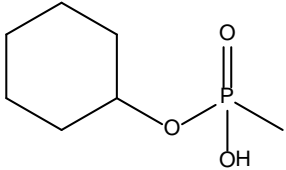
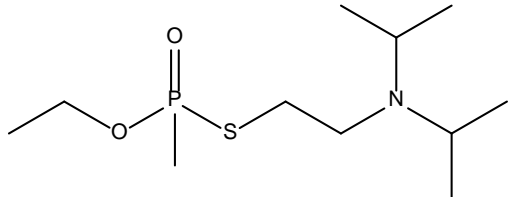
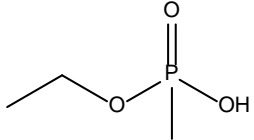
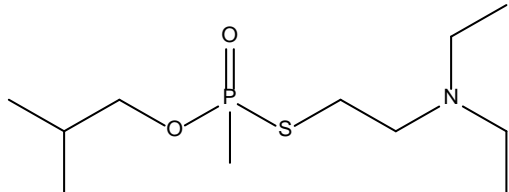
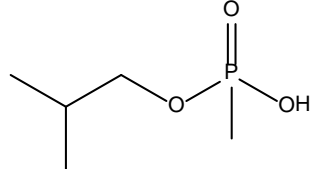
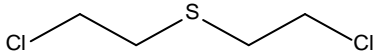
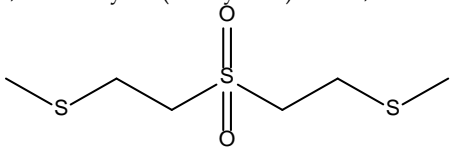
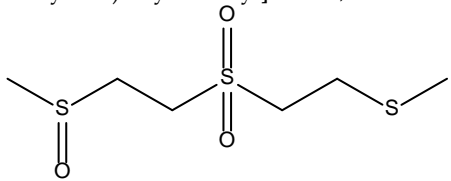
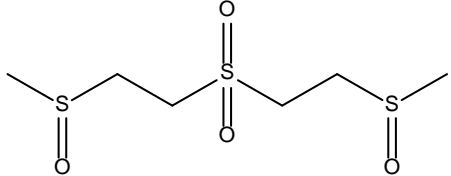
Agent	Metabolite
GA, Tabun 	Ethyl Dimethylphosphoroamidate, EDMAP, GA Acid 
GB, Sarin 	Isopropyl methylphosphonic acid, IMPA, GB Acid 
GD, Soman 	Pinacolyl methylphosphonic acid, PinMPA, GD Acid 
GF, Cyclo Sarin 	Cyclohexyl methylphosphonic acid, ChMPA, GF Acid 
VX 	Ethyl methylphosphonic acid, EMPA, VX Acid 
Russian VX, VR 	Isobutyl methylphosphonic acid, iBMPA, VR acid 

Table 2: Sulfur mustard β -Lyase metabolites

Agent	Metabolite
H, HD, Sulfur mustard, Mustard gas 	1,1'-sulfonylbis(methylthio)ethane, SBMTE 
	1-methylsulfinyl-2-[2-(methylthio)ethylsulfonyl]ethane, MSMTESE 
	1,1'-sulfonylbis[2-(methylsulfinyl)ethane], SBMSE 

2. Methods

2.1 Nerve agent metabolites, alkylphosphonic acids

The alkyl methylphosphonic acids examined in this study are water soluble. Attempts to separate mixtures of these compounds in aqueous solutions using reverse phase UHPLC fail to sufficiently resolve some of the lower molecular weight compounds. As an alternative, hydrophobic interaction liquid chromatography (HILIC) was employed. The use of HILIC to separate CWA degradation products has been previously described [2] but had not been applied to urinary metabolites. The HILIC-UHPLC conditions for the analysis required the sample be prepared in 5 mM ammonium formate, 95% acetonitrile (ACN)/water at pH 9 (Buffer B). Therefore, urinary samples require a solvent exchange prior to analysis. Five methods for performing solvent exchange were examined.

2.1.1 Sample preparation

Synthetic urine was prepared by adding 0.5 g of glucose, 5.0 g of NaCl and 2.0 g of urea to 500 mL of HPLC grade water.

The simplest sample preparation methods are those that involve the removal of the water in the sample. A comparison of five sample preparation methods was carried out in triplicate.

Method 1 - Lyophilisation

An aliquot (1 mL) of the sample was placed in a 20 mL vial and frozen with liquid nitrogen. A cover of aluminium foil with small holes in the foil was placed over the top of the vial. The vial was then placed in a freeze drying unit overnight to remove water by lyophilisation.

Method 2 - Evaporation

An aliquot (1 mL) of the sample was placed in a 20 mL vial and evaporated to dryness under a stream of dry nitrogen. The sample was heated to 80 °C during the evaporation. The time taken for evaporation of the sample was approximately five minutes.

Method 3 - Dual stage lyophilisation/evaporation

This is a combination of Method 1 and Method 2. The sample was resuspended in Buffer B (1 mL) prior to the commencement of Method 2.

Method 4 - Dual stage evaporation/evaporation

An aliquot (1 mL) of the sample was placed in a 20 mL vial and evaporated to dryness under a stream of dry nitrogen. After the evaporation the sample was resuspended in Buffer B (1 mL). The sample was agitated to suspend as much of the sample as possible then evaporated to dryness under a stream of dry nitrogen. During both evaporation steps the sample was heated to 80 °C.

Method 5 - Solid Phase Extraction (SPE)

SPE may be necessary if there is significant interference from other compounds in the urine to either the UHPLC or mass spectrometry.

An Env+ (300 mg) SPE cartridge was conditioned according to the manufacturers specifications (washed twice with 1 mL ACN, twice with 1 mL water). The sample (1 mL) was loaded onto the conditioned SPE cartridge. After the sample loading was complete the SPE cartridge was washed twice with 1 mL of water then eluted with 1 mL of ACN. The eluent was dried under a stream of dry nitrogen. The total time taken for sample preparation was approximately 30 minutes.

The dry samples from methods 1 to 5 were resuspended in 100 µL of Buffer B. In some cases the sample did not completely redissolve. An aliquot of the liquid portion was then analysed by UHPLC-MS/MS. Because some solid was carried over into the aliquot the samples were allowed to settle for at least 30 minutes before analysis, there was insufficient volume to filter the samples. The autosampler was set to sample above the bottom of the vial to further reduce sampling any suspended solid.

2.1.2 Instrument method

An Agilent 1290 Infinity UHPLC system with a Waters Acquity UPLC BEH HILIC 1.7 µm (2.1 mm x 150 mm) column combined with an Agilent 6460 triple quadrupole (QQQ) mass spectrometer equipped with an Agilent Jetstream source was used for the analysis.

Initially the chromatography conditions used were those employed during the 2009 OPCW biomedical exercise [1] the mass spectrometer conditions are similar to those listed in Table 4. The major changes investigated were to the gradient programme and the flowrate.

A solution of six alkyl methylphosphonic acids (EDMAP, IMPA iBuMPA, PinMPA, EMPA and MPA) was prepared at six concentrations spanning 16.7 ppm to 167 ppt (all were tenfold dilutions). The 1.67 ppm solution was then analysed using the same method, altering the flowrate in 0.5 mL/min steps from 0.5 mL/min to 2.0 mL/min.

The MRM chromatograms of these were examined and a flowrate of 1.0 mL/min was determined to be optimal.

Changes were also made to the gradient programme, reducing the time for a complete analysis cycle from 9 minute to 5 minutes. Gradient profiles are listed in Table 3.

Table 3: Changes to gradient profile

Initial setting	Final setting
Buffer A: 5 mM ammonium formate in water	Buffer A: 5 mM ammonium formate in water
Buffer B: 5 mM ammonium formate in 95% Acetonitrile 5% Water	Buffer B: 5 mM ammonium formate in 95% Acetonitrile 5% Water
0 to 4 min: 95%B to 85%B 4 to 4.1 min: 75%B to 30%B 4.1 to 6 min: 30%B 6 to 6.1 min: 30%B to 95%B 6.1 to 9 min: 95%B	0 to 3 min: 100%B to 90%B 3 to 4 min: 90%B to 30%B 4.0 to 4.1 min: 30%B to 100%B 4.1 to 5 min: 100%B
Flowrate 0.5 mL/min	Flowrate 1.0 mL/min

Optimised instrument parameters are listed in Table 4. The collision and fragmentor voltages were optimised for each analyte separately.

Table 4: UHPLC-MS/MS instrument settings

Instrument setting	Value
Column temperature	65 °C
Gradient programme	Solvent A: 5 mM ammonium formate in water (pH=9) Solvent B: 5 mM ammonium formate in 95% acetonitrile 5% Water (pH=9) 100 to 90% B (0.0-3.0 min), 90 to 30% B (3.0-4.0 min), 30 to 100% B (4.0-4.1 min), 100%B (4.1-5.0 min)
Flow rate	1.0 mL/min
Injection type	Agilent 1290 Autosampler
Injection volume	20 µL
Ionization	XESI (negative ion)
Spray voltage	3.5 kV
Capillary temperature	325 °C
Nebuliser gas and pressure	Nitrogen @ 20 psi
Drying gas and flow	Nitrogen @ 10 L/min

Sheath gas temperature/flow	400 °C / 11 L/min
Fragmentor voltage	110 V
Scan mode	Multiple reaction monitoring
Transitions monitored	PinMPA m/z 179 (M-H ⁺) → m/z 95; m/z 95 (M-H ⁺) → m/z 79 ChMPA m/z 177 (M-H ⁺) → m/z 95 m/z 95 (M-H ⁺) → m/z 79 EDMAP m/z 152 (M-H ⁺) → m/z 79 no second transition was observed IBMPA m/z 151 (M-H ⁺) → m/z 95 m/z 95 (M-H ⁺) → m/z 79 IMPA m/z 137 (M-H ⁺) → m/z 95 m/z 95 (M-H ⁺) → m/z 79 EMPA m/z 123 (M-H ⁺) → m/z 95 m/z 95 (M-H ⁺) → m/z 79
Scan time	0.6 s
Collision Voltage	15 V (PinMPA, CHMPA, IBMPA), 10 V (EDMAP, IMPA, EMPA)
Collision gas	Nitrogen @ 0.8 mTorr
Resolution	0.7 Da fwhm, MRM scan width 0.7 Da fwhm

2.2 β -Lyase metabolites of sulfur mustard

2.2.1 Sample preparation

The analysis of the β -lyase metabolites from synthetic urine utilised reverse phase UHPLC.. In order to obtain the fastest possible sample analysis no sample preparation was performed as the sample matrix was suitable for direct injection onto the column. A 100 μ L aliquot was taken from the sample and analysed by UHPLC-MS/MS.

2.2.2 Instrumental method

An Agilent 1290 Infinity UHPLC system with a Waters Acquity UPLC HSS T3 1.8 μ m (2.1 mm x 150 mm) column combined with an Agilent 6460 triple quadrupole (QQQ) mass spectrometer was used for the analysis. Instrument parameters are listed in Table 5.

Table 5: UHPLC/MSMS instrument settings for the analysis of β -lyase metabolites

Instrument setting	Value
Column Temperature	25 °C
Gradient programme	Solvent A: 0.05% Formic Acid in water Solvent B: 0.05% Formic Acid in Acetonitrile 0% B (0-2 min) to 70% B (2-12 min), 70% B (12-14 min) to 0% B (14.1-16 min)
Flow rate	0.5 mL/min
Injection type	Agilent 1290 Autosampler
Injection volume	20 μ L
Ionization	XESI (positive ion)
Spray voltage	3.5 kV
Capillary temperature	325 °C
Nebuliser gas and pressure	Nitrogen @ 20 psi
Drying gas and flow	Nitrogen 10 L/min
Sheath gas temperature/flow	400 °C / 11 L/min
Fragmentor voltage	110 V
Scan mode	Multiple reaction monitoring
Transitions monitored	Bis-sulfoxide (SBMSE) m/z 269 (MNa ⁺) \rightarrow m/z 205 m/z 269 (MNa ⁺) \rightarrow m/z 141 (confirmation) Mono-sulfoxide (MSMTESE) m/z 253 (MNa ⁺) \rightarrow m/z 189 m/z 253 (MNa ⁺) \rightarrow m/z 75 (confirmation) Bis-sulfide (SBMTE) m/z 237 (MNa ⁺) \rightarrow m/z 75 no second transition was observed
Scan time	0.6 s
Collision Voltage	10 V (SBMSE, MSMTESE), 12 V (SBMTE)
Collision gas	Nitrogen 0.8 mTorr
Resolution	0.7 Da fwhm, MRM scan width 0.7 Da fwhm

Transitions from the sodium adduct ions were used as these were the most abundant molecular ion species.

3. Results

3.1 Alkylphosphonic acid analysis

3.1.1 Effect of flowrate on the separation of alkyl methylphosphonic acids

Figure 2 shows the changes to the separation of the analytes due to alteration of flowrate from 0.5 mL/min to 2.0 mL/min.

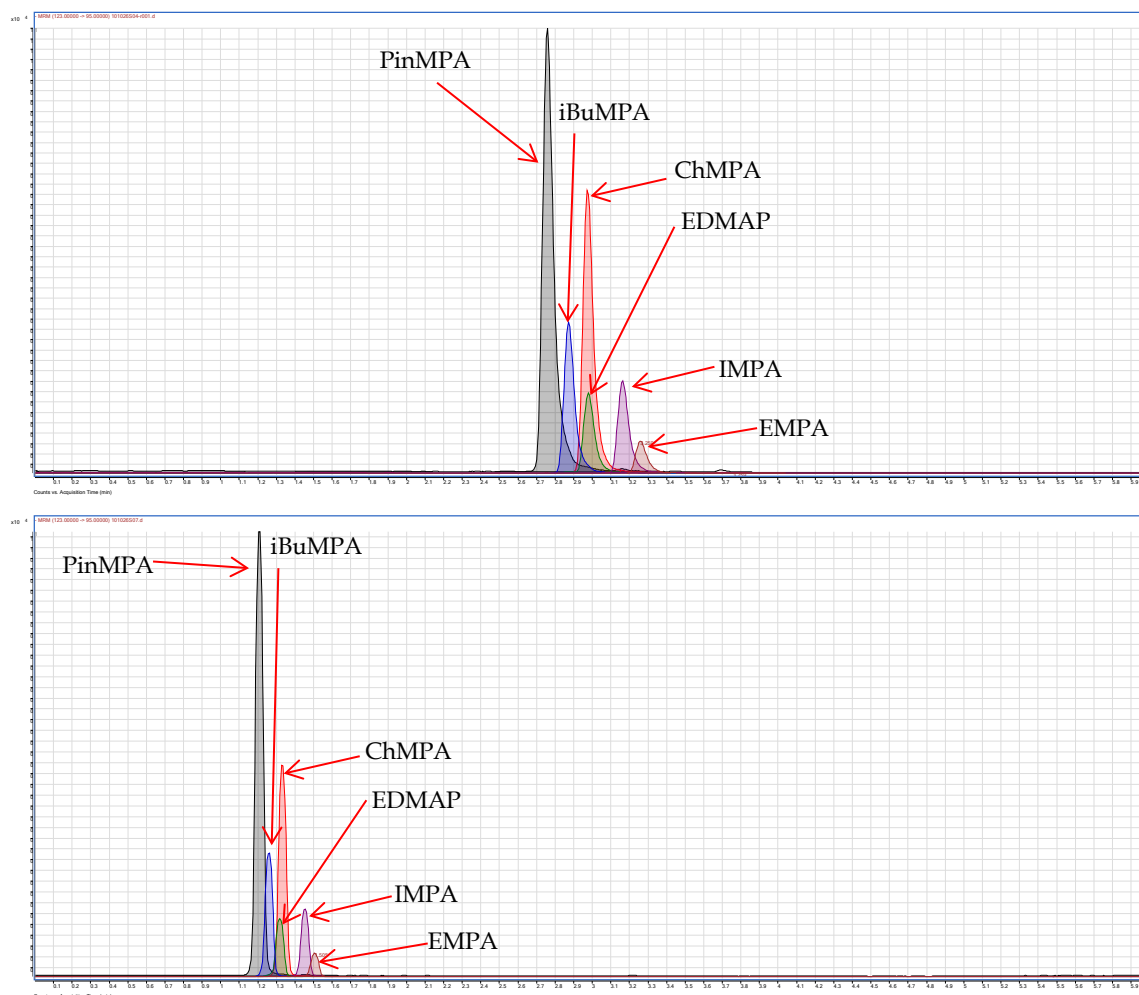


Figure 1: UHPLC-MS/MS EIC for the MRM transitions m/z 179→ m/z 95 (PinMPA), m/z 151→ m/z 95 (iBMPA), m/z 177→ m/z 95 (ChMPA), m/z 152→ m/z 79 (EDMAP), m/z 137→ m/z 95 (IMPA) and m/z 123→ m/z 95 (EMPA) at 0.5 mL/min (upper) and 2.0 mL/min (lower)

There was little change in the amount of overlap between analytes; in particular the peaks for EDMAP and ChMPA completely overlap regardless of the flowrate used.

There was significant change to the peak shape, peak height and integral with a change in flowrate. Figure 2 shows the variation of the PinMPA peak with the four flowrates tested.

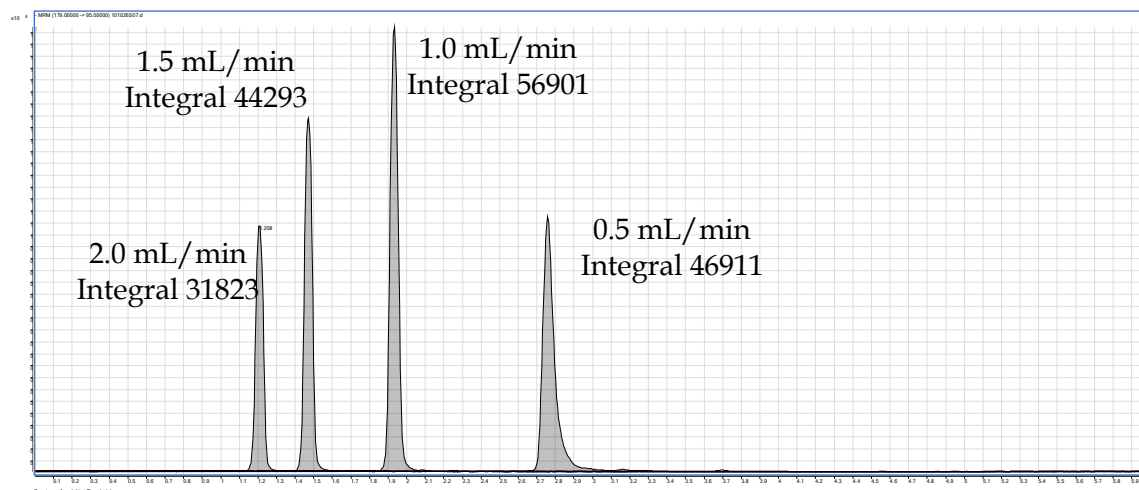


Figure 2: EIC of PinMPA at four different flowrates

The response at a flowrate of 1.0 mL/min was optimal and the peak shape and resolution were also good. This flowrate was therefore used for the remainder of the experiments.

3.1.2 Standard curve generation and determination of LOD and LOQ.

A typical chromatogram from the analysis of a mixture of alkylphosphonic acids is shown in Figure 3. The sample consisted of a mixture of the six metabolic products listed in Table 1 at a concentration of 1.67 ppm. The chromatography shows good separation for all but two of the compounds, cyclohexyl methylphosphonic acid (ChMPA) and ethyl dimethylphosphoroamidate (EDMAP). The MRM transitions allow the data from these two compounds to be distinguished as there is no crossover between the two transitions.

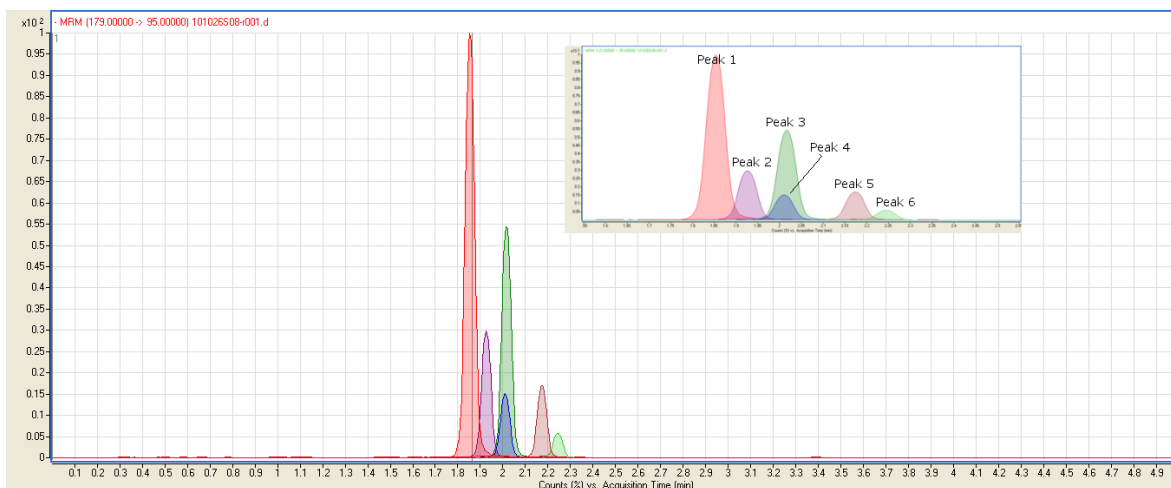


Figure 3: UHPLC-MS/MS EIC for the MRM transitions m/z 179 \rightarrow m/z 95 (PinMPA, Peak 1), m/z 151 \rightarrow m/z 95 (iBMPA, Peak 2), m/z 177 \rightarrow m/z 95 (ChMPA, Peak 3), m/z 152 \rightarrow m/z 79 (EDMAP, Peak 4), m/z 137 \rightarrow m/z 95 (IMPA, Peak 5) and m/z 123 \rightarrow m/z 95 (EMPA, Peak 6)

Calibration curves for each of the alkylphosphonic acids (with the exception of ethyl methyl phosphonic acid) were generated from a series of seven standards in the concentration range of 0.5 ppb to 50 ppb in acetonitrile. An internal standard, deuterated IMPA (d_7 , 10 ppb), was added to the acetonitrile used to prepare the standard solutions. The peaks corresponding to the alkylphosphonic acid MRM transitions were extracted and integrated. The area corresponding to each peak was divided by the area corresponding to that of the internal standard to give the Area Ratio (AR). The AR was then plotted against the concentration of the standard and a linear regression analysis carried out. Figure 4 shows the calibration curves for each of the alkylphosphonic acids.

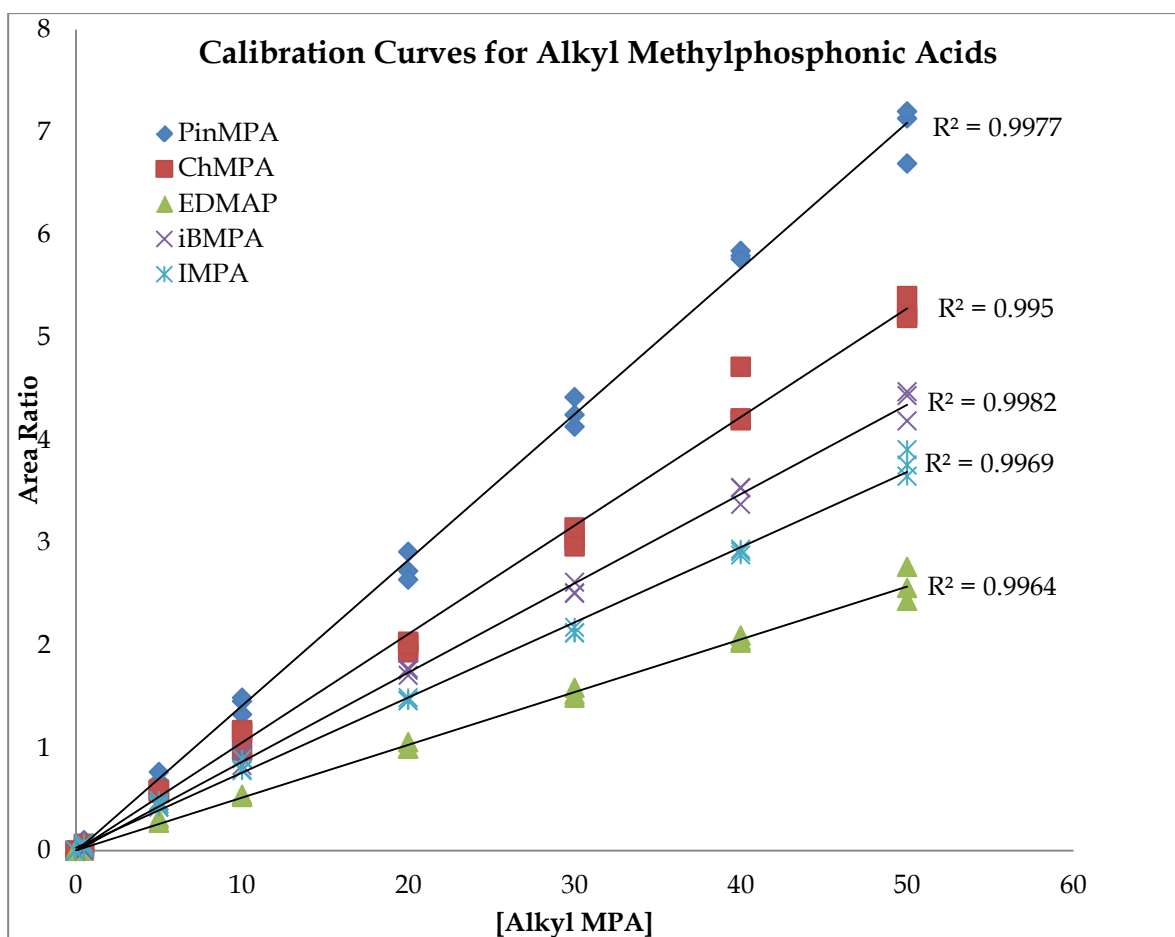


Figure 4: Calibration curves for alkyl methylphosphonic acids

The LOD and LOQ values were estimated from the linear regression statistics using the STEYX and SLOPE functions of Microsoft Excel to estimate the residual standard deviation of the regression and the slope of the calibration curve respectively. The LOD and LOQ values were then calculated using the following formulae and summarised in Table 6;

$$LOD = \frac{3.3 * STEYX}{SLOPE}$$

$$LOQ = \frac{10 * STEYX}{SLOPE}$$

Table 6: Limits of detection and quantitation for the alkylphosphonic acids

Compound	LOD (ppb)	LOQ (ppb)
PinMPA	2.6	8.8
ChMPA	3.9	13.1
EDMAP	3.4	11.2
iBMPA	2.4	7.9
IMP	3.1	10.2

3.1.3 Effect of sample preparation method on the amount of analyte recovered from synthetic urine

A typical chromatogram for alkyl methylphosphonic acids extracted from synthetic urine is shown in Figure 5. Note that there is little change between the retention times of the analytes between this sample and the aqueous sample (Figure 3).

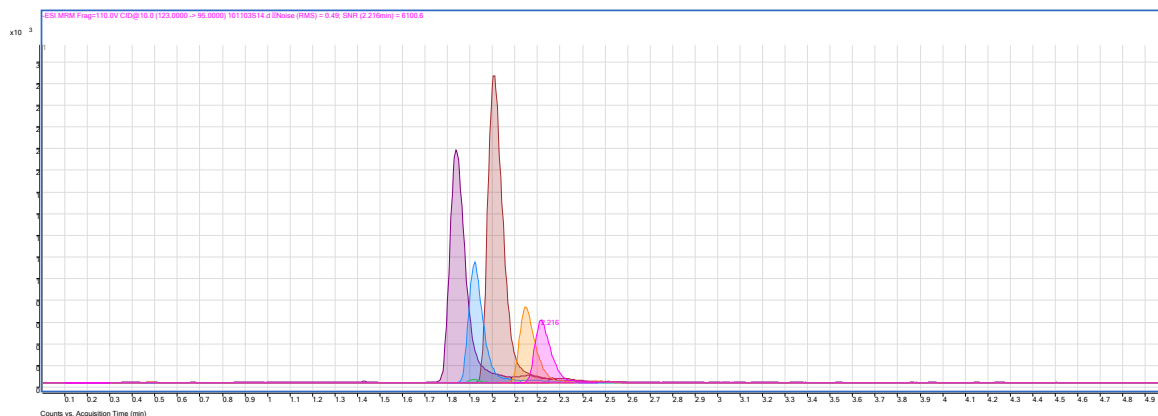


Figure 5: UHPLC-MS/MS EIC for the MRM transitions m/z 179 \rightarrow m/z 95 (PinMPA, Peak 1), m/z 151 \rightarrow m/z 95 (iBMPA, Peak 2), m/z 177 \rightarrow m/z 95 (ChMPA, Peak 3), m/z 137 \rightarrow m/z 95 (IMPA, Peak 4) and m/z 123 \rightarrow m/z 95 (EMPA, Peak 5)

Samples contained known amounts of agent metabolite in both water and synthetic urine matrices. Figure 6 shows the percentage of the metabolite recovered from water samples. This is calculated from the ratio of the integral of the extracted peak for the MRM transition for each compound versus that of a standard of equivalent concentration. This represents the maximum amount of each analyte that could possibly be recovered under ideal conditions with little or no influence of the matrix.

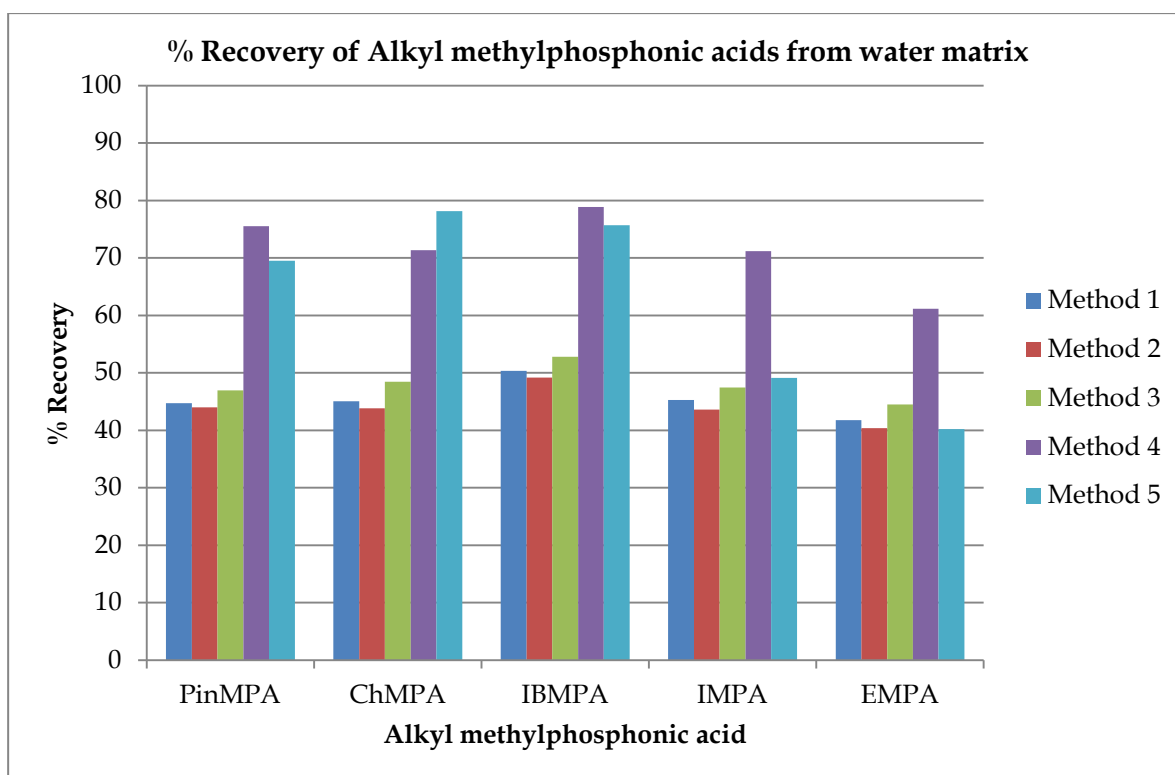


Figure 6: Recovery of nerve agent metabolites from a water matrix

Figure 7 shows a similar recovery calculation from samples using a synthetic urine matrix.

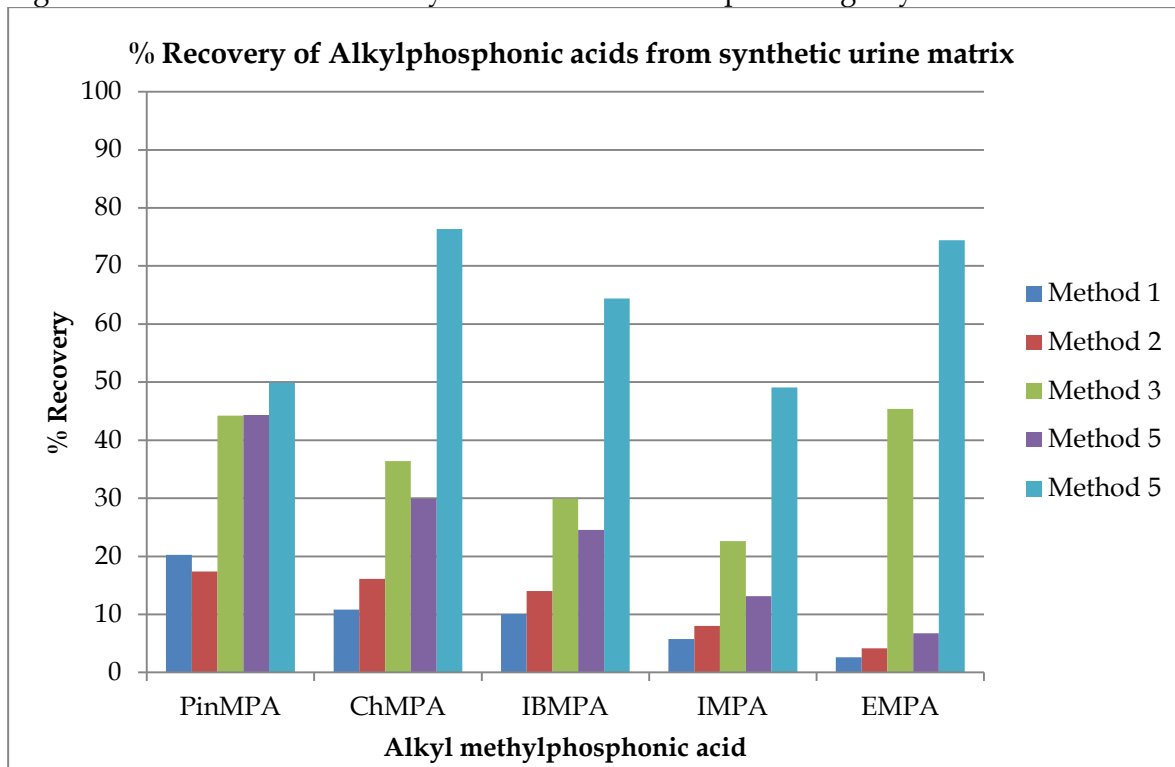


Figure 7: Recovery of nerve agent metabolites from a urine matrix

The amount of analyte recovered from the synthetic urine samples was generally lower than that for samples in a water matrix. There was some influence of the compound molecular weight, with lower molecular weight species having lower recovery from the synthetic urine samples. In general the recovery followed the order of PinMPA>ChMAP>iBuMPA>IMPA>EMPA, which is also the molecular weight order 179 Da>177 Da>151 Da>137 Da>123 Da, whereas the hydrophobicity (which can be established relatively from the elution order) follows the order PinMPA>iBuMPA>ChMPA≈IMPA>EMPA.

3.2 β -Lyase metabolites of sulfur mustard

A typical chromatogram from the analysis of a mixture of β -lyase metabolites in water is shown in Figure 8.

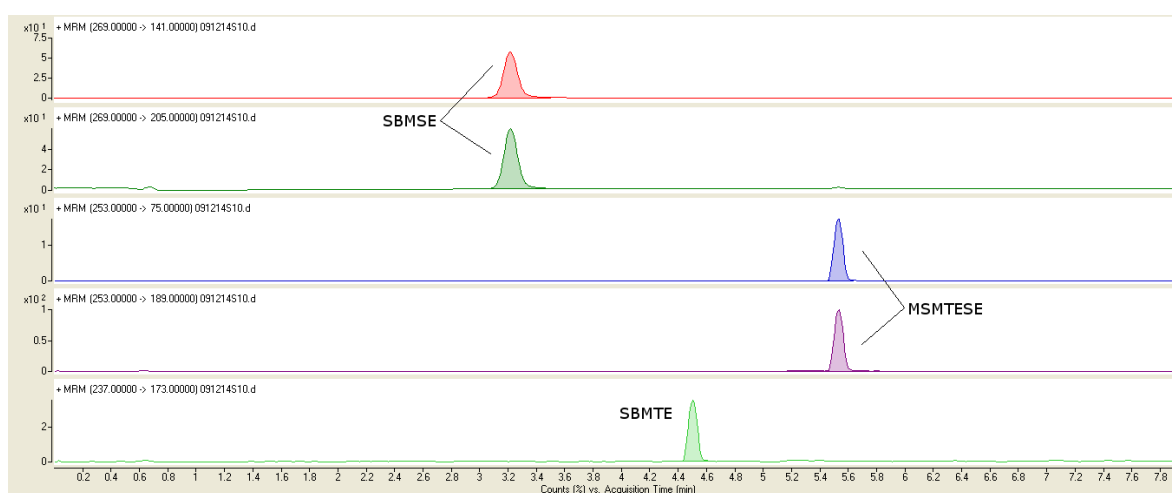


Figure 8: LC-MS-MS EIC for in house reference samples. The transitions shown are m/z 269 \rightarrow 141 and m/z 269 \rightarrow 205 for SBMSE, m/z 253 \rightarrow 75 and m/z 253 \rightarrow 189 for MSMTESE and m/z 237 \rightarrow 173 for SBMTE.

After completion of the 2009 OPCW confidence building exercise, the OPCW released the identity and concentrations of the spiking chemicals in the samples provided. An estimate of the efficiency of the analysis method was made by comparison of the ratio of the integrals of the MRM chromatograms for the synthetic urine samples supplied by the OPCW to standards at equivalent concentrations in a water matrix. Taking both the m/z 269 \rightarrow 205 and the m/z 269 \rightarrow 141 transitions into account the efficiency of the analysis method was in the order of 90%. The determination of the LOD and LOQ for these metabolites was not performed due to the lack of a suitable isotopically labelled internal standard.

4. Conclusions

Sample preparation and UHPLC-MS/MS methods have been developed for the rapid analysis of selected urinary metabolites of nerve agents and sulfur mustard.

The instrument methods are both rapid (total run time between samples is eight minutes) and sensitive. The LOD for the metabolites of nerve agents was in the range of 2 ppb to 4 ppb, the LOQ was 8 ppb to 14 ppb. These results are consistent with results obtained in the laboratory during an OPCW confidence building exercise held in December 2009. The LOD and LOQ for the sulfur mustard metabolites were not determined, though during the OPCW exercise we were able to detect SBMSE standards down to high ppt levels.

The sample preparation methods for the nerve agent metabolites are all quite simple, though the speed of analysis varies greatly from a matter about 10 minutes to overnight. The amount recovered also depends greatly on the metabolite in question. When combined with the amount recovered for each agent metabolite the method limits of detection varied from approximately 3 ppb to 67 ppb and the limit of quantitation from approximately 10 ppb to 270 ppb, depending on which sample preparation method was used.

Of the sample preparation methods investigated, method 5 (SPE using ENV+) clearly performed the best in terms of recovery of agent metabolites from synthetic urine. The recovery for all analytes was greater than 49% and, although more complex, the sample preparation method was less than 20 minutes. The resultant total analysis time should be approximately 30 minutes.

The sample preparation method chosen for alkyl methylphosphonic acids in urine will depend on a number of factors. The time available before a result from the analysis is required and the expected amount of agent metabolite that would be present are the major factors involved in making the decision. If there is enough time (i.e. more than 30 minutes) the more sensitive Method 5 (SPE) should be used as the recoveries of all alkyl methylphosphonic acids was over 49 %, however if the analysis time needs to be less than 30 minutes and the amount of metabolite is suspected to be well above the LOD, then the more rapid and simple single stage evaporation (Method 1) would be suitable.

As the nerve agent metabolites are generally the same as the hydrolysis product for that particular nerve agent [3], these HILIC methods could also find application in the analysis of environmental water samples.

The analysis of mustard metabolites showed some variation between matrices of the relative amounts of fragment ions. This could be due to many factors and requires further investigation to exclude interfering compounds.

5. References:

- [1] Report of the First Confidence Building Exercise for Biomedical Sample Analysis, J. Doward, B Muir, A Theo and S. Thomson, 2009 (Unpublished report).
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19. ABSTRACT This report outlines the instrumental method development for the analysis of human biofluids, in this case urine, for the presence of metabolites of the chemical warfare agents HD, GA, GB, GD, GE, GF, VX and VR using UHPLC MSMS. It also investigates the effect of different sample preparation techniques on the recovery of the analytes, using the analysis method developed.					